

Diesel Exhaust Particles Activate the Matrix-Metalloproteinase-1 Gene in Human Bronchial Epithelia in a β -Arrestin-Dependent Manner via Activation of RAS

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SUPPLEMENTAL MATERIAL

Supplementary Figures

Figure 1: MMP-1 Western blotting on BEAS-2B cell protein extract. The left-hand side panel shows the blot of protein extract from BEAS-2B cells, 10 µg total protein per lane, for control stimulation using media, P90 carbon control particles vs. DEP (100 µg/mL). The right-hand side panel shows densitometric evaluation of relative protein abundance of MMP-1, normalized for β-actin, for n=3 samples, with increased expression for DEP stimulation; * denotes statistically significant increase vs. controls (p<0.05).

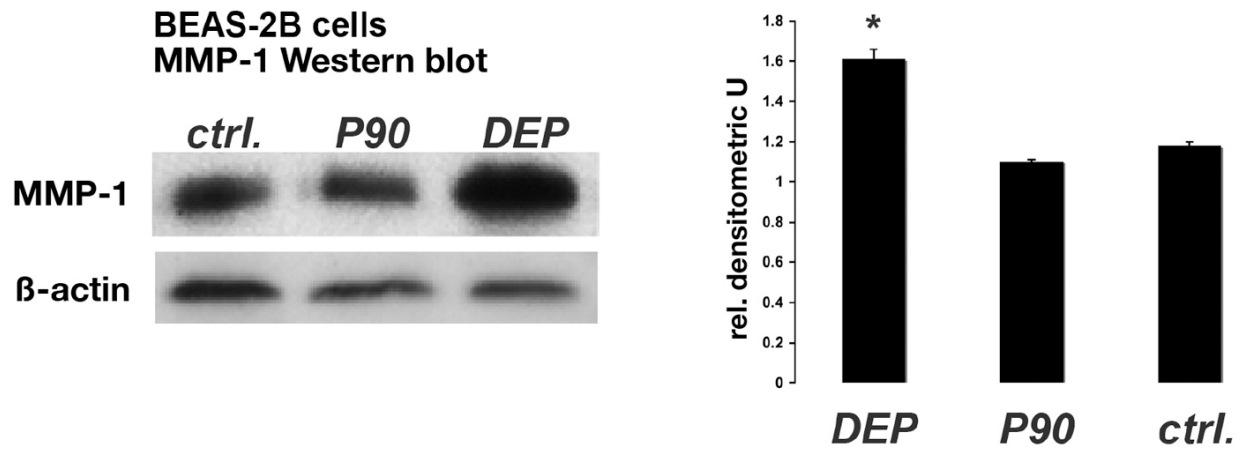
Figure 2: Secreted MMP-1 protein, as measured by ELISA, and specific MMP-1 cleavage activity, correlate significantly. MMP-1 cleavage activity was measured using a commercially available assay, see Methods. Upon specific MMP-1 cleavage, a FRET signal can be monitored, correlating well with secreted amounts of MMP-1.

Figure 3: *MMP-1* promoter driven reporter genes, depicted above, illustrate that the -1607GG polymorphism is associated with a robustly increased transcriptional activation, depicted in bar diagrams below. The strongest activation is observed for the 2.9kB promoter, with longer promoters reducing activity. – For a thorough bio-informatic analysis of the “tobacco-response element”, -4.4 to -2.9kB, see (Mercer et al. 2008).

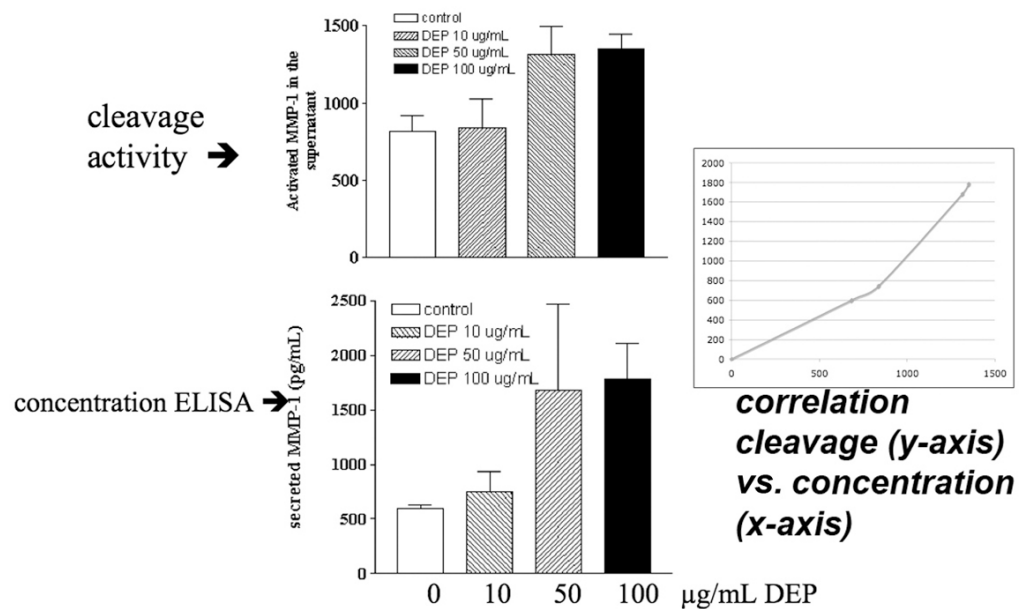
Figure 4: Specific inhibition of p38 and JNK MAP-kinases, using SB203580 and SP600125, do not down-regulate activation of the *MMP-1* gene in BEAS-2B cells; reporter gene assays for both -1607 polymorphisms is shown in the upper panel, MMP-1 secretion below. For specific inhibition of JNK, reporter-gene activity is significantly increased for the 1G polymorphism in response to DEP (100µg/mL), secreted MMP-1 is higher, but this increase does not reach the level of statistically significant difference.

Figure 5: A time-course of phospho-ERK immunofluorescence in response to DEP stimulation is depicted, using confocal laser-scanning microscopy. A peak of the nuclear signal for the 30' time-point is apparent, at which time-point phospho-ERK and phospho-MEK Western blotting has been conducted, depicted below.

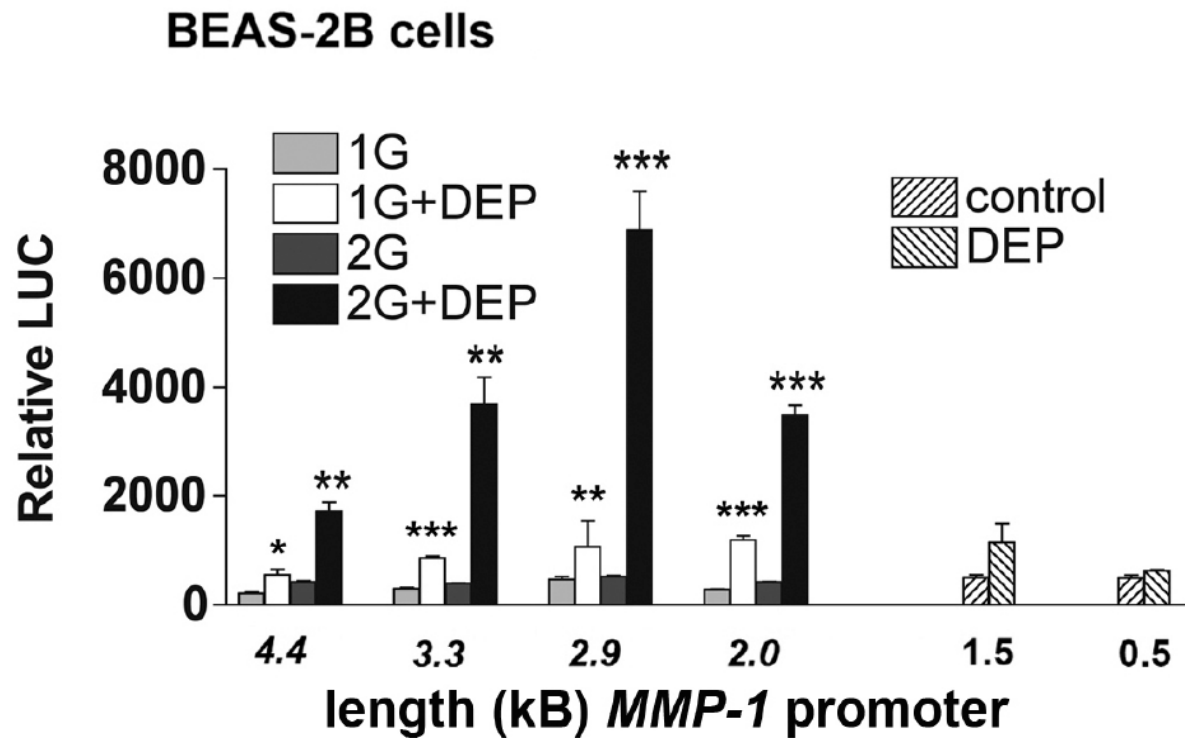
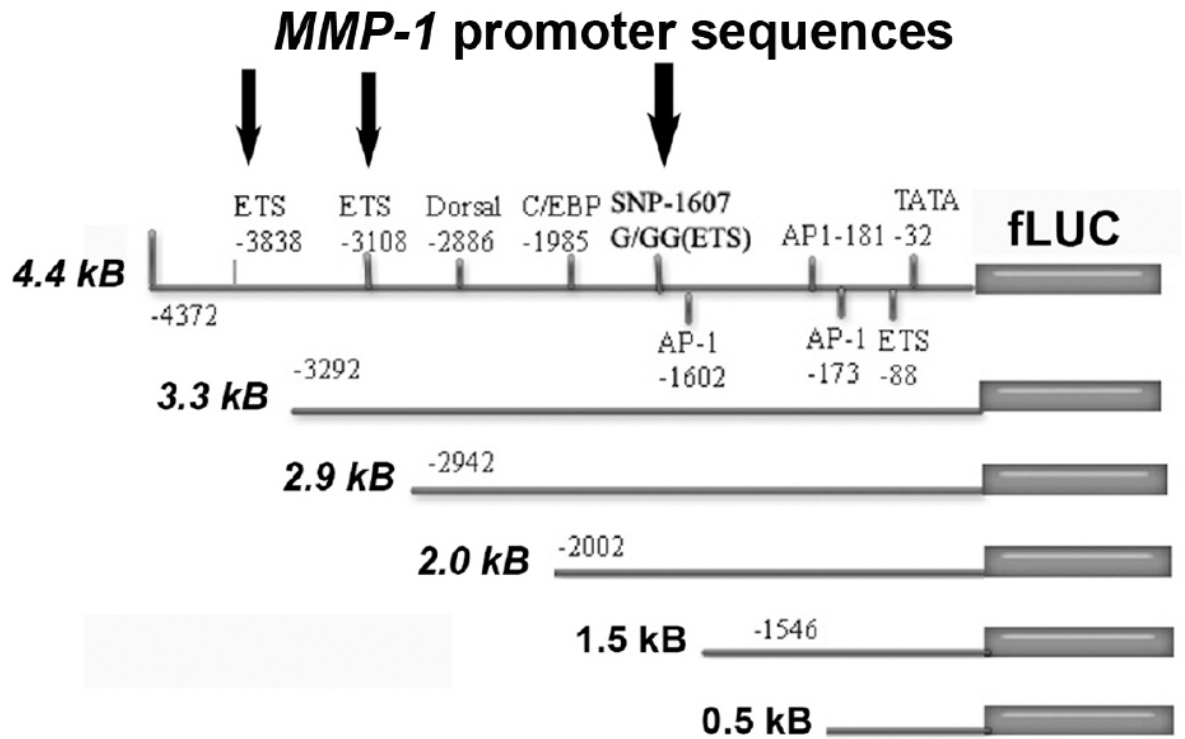
Supplementary Material, Figure 1



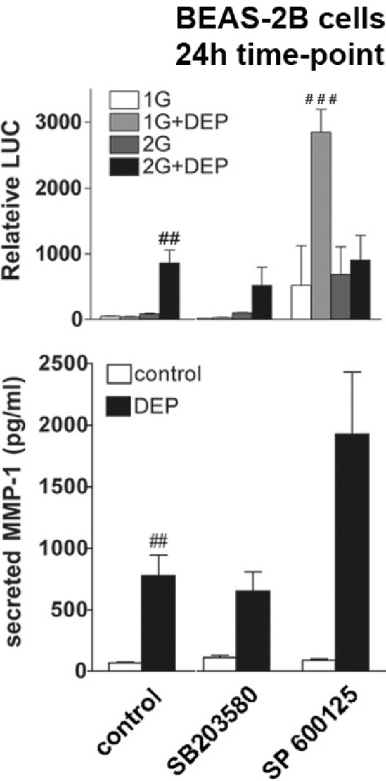
Supplementary Material, Figure 2



Supplementary Material, Figure 3



Supplementary Material, Figure 4



Supplementary Material, Figure 5

anti-phospho-ERK immunocytochemistry, confocal laser scanning

